The Examiner is contending that there is no support in the specification for altering amino acids within the range of amino acid 18-28, 55-65, 62-72, 86-96, 89-99 and 205-215. Recognizing that we have based these ranges as being within five amino acids of a particular amino acid (such as 23), the Examiner is arguing that the specification only supports the ± 5 amino acid concept for the specific amino acid positions, presumably those disclosed on page 19, line 22 – page 20, line 2 (i.e., positions L45, E67, Y89, or Y115 of SEB).

To that end, we first note the Examiner included the range of 62-72 as non-supported, although it is within this list as E67.

We point out that the specification at page 20, line 24 – page 21, line 17 provides a description of the amino acid positions of N23 (± 5 range 18-23), N60 (± 5 range 55-65), Y91 (± 5 range 86-96), and D210 (± 5 range 205-210). The description explicitly specifies that the residues can be altered within 5 amino acids of these positions. The Examiner indicated that these specific ranges are not disclosed on the pages we referred to in our April 10, 2002 Preliminary Amendment. We speculate that, for some reason, the copy of the specification in the Examiner's file for this case has differing page numbering from our copy of the specification. To that end, appended here is a copy of pages 20 and 21 of the specification that is in our files, and that, to the best of our knowledge, is correctly page-numbered.

Regarding claim 110, we have amended this claim above to address the Examiner's concerns as specified in the Office Action.

Reconsideration of these objections are therefore requested.

Claims 21-23 are rejected under 35 U.S.C. §112, second paragraph. We have amended these claims above to address the Examiner's concerns as specified in the Office Action. Reconsideration of this objection is therefore requested.

In summary, it is submitted that the language of all the pending claims is fully supported by the disclosure as originally filed, and withdrawal of this rejection is believed to be in order. All of the Examiner's outstanding rejections and objections have been addressed, and the application is believed to be in allowable form. Notice to that effect is earnestly solicited. No amendment made was related to the statutory

requirements of patentability unless expressly stated herein, and no amendment made was for the purpose of narrowing the scope of any claim unless we argued above that such amendment was made to distinguish over a particular reference or combination of references.

If the Examiner has any questions or would like to make suggestions as to claim language, she is encouraged to contact Marlana K. Titus at (301) 924-9600.

Respectfully submitted,
By

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MARKED-UP VERSION OF AMENDED CLAIMS

21. (Thrice amended) A recombinant DNA construct comprising a vector [and] containing an isolated and purified superantigen toxin DNA fragment encoding Staphylococcal enterotoxin B (SEB), wherein said DNA fragment has the sequence according to sequence of SEQ ID NO:5.

22. (Thrice amended) A recombinant DNA construct comprising a vector [and] containing an isolated and purified superantigen toxin DNA fragment encoding Staphylococcal enterotoxin B (SEB), wherein said DNA fragment has the sequence according to sequence of SEQ ID NO:7.

23. (Thrice amended) A recombinant DNA construct comprising a vector [and] containing an isolated and purified superantigen toxin DNA fragment encoding Staphylococcal enterotoxin B (SEB), wherein said DNA fragment has the sequence according to sequence of SEQ ID NO:9.

110. (Amended) An isolated and purified superantigen toxin DNA fragment encoding Staphylococcal enterotoxin B (SEB), wherein said DNA fragment comprises the sequences according to sequence of SEQ ID NO:5 and SEQ ID:NO 6, in which Staphylococcal enterotoxin B the amino acids at positions 18-28, 55-65, and 205-215 of SEB have been altered such that binding of said encoded SEB to the MHC class II receptor and T cell antigen receptor is altered.

pocket that can contact HLA-DR, (such as E67, Y89, or Y115 of SEB). The residues can be changed or substituted to alanine for minimal disruption of protein structure, more preferably to a residue of opposite chemical characterisitcs, such as hydrophobic to hydrophilic, acidic to neutral amide, most preferably by introduction of a residue with a large hydrated side chain such as Arginine or Lysine. In addition, side chains of certain nonconserved receptor-binding surfaces, can also be altered when designing superantigen toxins with low binding affinities. These residues can include Y94 of SEB and structurally equivalent residues of other superantigens, such as A97 of SEA, or any side chain within 5 residues from these positions or any side chain in discontinuous positions (discontinuous positions are defined as amino acid residues that fold together to form part of a discrete three-dimensional structural unit but are not present on the same secondary structural unit e.g. α helix or β-strand) such as disulfide-bonded side chains, that involve, directly or indirectly, the nonconserved receptor contact surfaces outside of the polar binding pocket or hydrophobic loop. Further, amino acid residues involved with protein folding or packing can be altered when designing superantigen toxins with low binding affinities [Sundstrom *et al.* (1996) *EMBO J.* **15**, 6832-6840; Sundstrom *et al.* (1996) *J. Biol. Chem.* **271**, 32212-32216; Acharya *et al.* (1994) *Nature* **367**, 94-97; Prasad *et al.* (1993) *Biochem.* **32**, 13761-13766; Swaminathan *et al.* (1992) *Nature* **359**, 801-806]. Furthermore, especially for superantigens with higher affinities for T-cell antigen receptors, side chains of amino acids within 5 residues of the position represented by N23 (conserved residue in most

superantigens) , N60 (conserved Asn or Trp in most superantigens) Y91 (semiconserved hydrophobic residues Trp, Ile, Val, His in most superantigens) and D210 of SEB (conserved Asp in most superantigens) can be altered when designing superantigen toxins with low binding affinities. These residues are likely to form part of the integral molecular surfaces that are in contact with T-cell antigen receptors. Because the T-cell receptor contact areas of superantigen toxins are essential for causing specific activation or inactivation of T-cell subsets, altering residues that are unique to each superantigen but that are located within 5 residues of the positions represented by N23, N60 and Y91 can produce superantigens that affect a smaller number (e.g. 1-3) of subsets. Such altered superantigen toxins can be useful as therapeutic agents.

In another embodiment, the present invention relates to a DNA or cDNA segment which encodes a superantigen toxin such as SEA, SEB, SEC-1, SPEa, and TSST-1 to name a few, the sequence of which has been altered as described above to produce a toxin protein with altered binding ability to MHC Class II and/or T-cell receptors. For SEA, the following three mutations were introduced into the toxin molecule: Tyrosine at amino acid position 92 changed to alanine; Aspartic acid at amino acid position 70 changed to arginine; Leucine at amino acid position 48 changed to arginine. The reduction in binding to HLA DR is additive per mutation, though one or two mutations can produce a vaccine and a combination of all three mutations in one molecule produces a better vaccine. Other substitutions can also result in reduced binding.